Analysis of Antiepileptic Drugs by HPLC

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Abstract

Quantitative determination of antiepileptic drugs in biological fluids using HPLC is an important way to facilitate the individualization of dosage. For example, even minor adjustments in the daily dosage of phenytoin can greatly affect its steady-state concentration and the patient's concentration-dependent clearance.
Several methods are available for the quantitative determination of antiepileptic drugs. The most common and generally accepted ones are based on GC, HPLC and immunoassay technique. Figure 1 shows the HPLC chromatogram of eight common antiepileptic drugs using gradient analysis on a reversed phase column and UV detection. The autosampler temperature was set to 4 ºC to avoid decomposition of the samples.

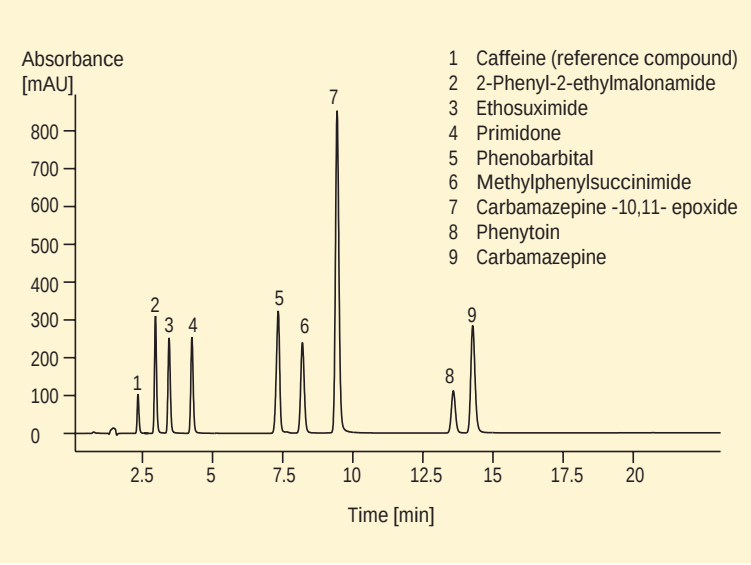

Figure 1
Analysis of eight antiepileptic drugs

Conditions

Column 4 x 125 mm ODS Hypersil 5 µm
Mobile phase
A = water
B = acetonitrile
Flow rate 0.8 ml/min
Gradient 15 % B to 40 % B in 25 min
Column wash
40 % B to 80 % B in 5 min
80 % B to 15 % B in 2 min
UV detector
diode array detector:
204/10 nm, reference 360/80 nm
standard cell
variable wavelength detector
204 nm, standard cell
Column compartment temperature
60 °C
Stop time 32 min
Post time 5 min
Injection volume 5 µl

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The performance of the HPLC method is shown in the table below.

Compound	LOD for S/N=2 (mg/l)*	Precision of RT (RSD of 20 runs (100 mg/l)*	Precision of Area (RSD of 20 runs) (100 mg/l)*	Linearity Correlation factor (0.1-100 mg/l)*
Phenobarbital	0.1	0.09	1.31	1.00000
2-Phenyl-2-ethylmalonamide	0.1	0.10	1.28	1.00000
Primidone	0.1	0.09	1.28	1.00000
Carbamazepine-10, 11-epoxide	0.1	0.10	1.31	1.00000
Carbamazepine	0.1	0.05	1.29	1.00000
Phenytoin	0.1	0.06	1.26	1.00000
Ethosuximide	0.1	0.08	1.25	0.99999
Methylphenylsuccinimide	0.1	0.06	1.26	1.00000
Caffeine (reference compound)	0.1	0.11	1.28	1.00000

* Injection volume: 5 µl

The HPLC method presented here shows an easy but reliable and precise analysis of the antiepileptic drugs 2-phenyl-2-ethylmalonamide, ethosuximide, primidone, phenobarbital, methylphenylsuccinimide, carbamazepine-10,11-epoxide, phenytoin, carbamazepine and caffeine as a reference compound. The values for LOD, precision of RT, precision of area and linearity show the good performance of the analysis.

Equipment

Agilent 1100 Series

- Quaternary pump (includes vacuum degasser)
- Thermostatted autosampler
- Thermostatted column compartment
- Diode-array detector, standard flow cell, 10-mm path length, 13-µl cell volume

Alternative:

- Vacuum degasser
- Binary pump
- Variable wavelength detector, standard flow cell 10-mm path length, 13-µl cell volume
- Agilent ChemStation + 3D software

Columns

- Hypersil ODS, 5 µm, 4 x 125 mm (Agilent part number 792618-564)
- *Recommended:* Guard cartridges Hypersil ODS, 5 µm 4 x 4 mm (Agilent part number 7992618-504, 10/pk)

Note:
Since the method was specifically developed on the Agilent 1100 Series system you might not be able to reproduce this analysis on an older system or even on a new system with lower performance. To avoid sample decomposition it is necessary to use a cooled autosampler, for example, the Agilent1100 Series thermostatted autosampler.

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